Journal of Chemical Ecology, Vol. 12, No. 6, 1986

EFFECTS OF CUTICULAR DUVANE DITERPENES FROM GREEN TOBACCO LEAVES ON TOBACCO BUDWORM (LEPIDOPTERA: NOCTUIDAE) OVIPOSITION[1]

D. MICHAEL JACKSON, R.F. SEVERSON, A.W. JOHNSON, AND G.A. HERZOG[2]

Tobacco Research Laboratory, Agricultural Research Service
U.S. Department of Agriculture
Oxford, North Carolina 27565

(Received February 11, 1985; accepted September 12, 1985)

Abstract—Five cuticular chemical components isolated from the green leaves of tobacco introductions (TIs) and a commercial tobacco cultivar were tested for their effects on tobacco budworm, *Heliothis virescens* (F), oviposition in cage bioassays, and field experiments. These chemicals were sprayed onto budworm-resistant TI 1112 tobacco which produces low levels of most cuticular components. Individual duvane diterpenes (α- and β-4,8,13-duvatrien-1-ols and α- and β-4,8,13-duvatriene-1,3-diols) increased tobacco budworm egg laying on sprayed TI 1112 plants. *cis*-Abienol, docosanol, and docosanyl myristate were inactive.

Key Words—Tobacco budworms, *Heliothis virescens* (F), Lepidoptera, Noctuidae, tobacco, *Nicotiana tabacum* L., diterpenes, duvanes, host plant resistance, oviposition.

INTRODUCTION

The leaves of commercially grown tobaccos, *Nicotiana tabacum* L., are covered by sticky exudates that are excreted onto the leaf surface from glanded

[1] Mention of a company or proprietary product does not imply endorsement or recommendation by the U.S. Department of Agriculture. Paper No. 9702 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, North Carolina 27695-7601.

[2] Tobacco Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Oxford, North Carolina 27565 (D.M.J.); Tobacco Safety Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Athens, Georgia 30613 (R.F.S.); Pee Dee Research and Education Center, Department of Entomology, Fisheries and Wildlife, Clemson University, Florence, South Carolina (A.W.J.); and Coastal Plain Experiment Station, Department of Entomology and Fisheries, University of Georgia, Tifton, Georgia 31794 (G.A.H.).

trichomes (Michie and Reid, 1968; Severson et al., 1984). The major cuticular components consist primarily of diterpenes (labdanes and duvanes), hydrocarbons, and sucrose esters (Enzell et al., 1977, Severson et al., 1984). *N. tabacum* varieties and tobacco introduction (TIs) produce primarily labdanes (some oriental and cigar tobaccos), primarily duvanes (many flue-cured and burley tobaccos), or a combination of both types of diterpenes (Reid, 1979, 1980; Sato et al., 1982; Severson et al., 1984). Tobaccos with simple (nonglanded) trichomes, such as TI 1112, produce only trace amounts of diterpenes and sucrose esters, and these tobaccos are resistant in the field to green peach aphids, *Myzus persicae* (Sulzer) (Johnson and Severson, 1982), and tobacco budworms, *Heliothis virescens* (F) (Elsey and Chaplin, 1978; Severson et al., 1983; Johnson and Severson, 1984), and tobacco hornworms, *Manduca sexta* (L.) (Chaplin et al., 1976, Johnson, 1978).

In controlled cage studies with tobacco budworm moths, Jackson et al. (1983) demonstrated that one mechanism of host plant resistance in TI 1112 is ovipositional nonpreference. The whole leaf wash (WLW) extracted from the budworm-susceptible commercial flue-cured variety, NC 2326, stimulated tobacco budworm oviposition when sprayed onto TI 1112 potted plants in cage and field experiments (Jackson et al., 1984). NC 2326 WLW was fractionated into a methanol–water-soluble (MWS) fraction, containing predominantly α- and β-4,8,13-duvatriene-1,3-diols (α- and β-diols), and a hexane-soluble (HS) fraction consisting of hydrocarbons, fatty alcohols, and wax esters. Only the MWS fraction stimulated tobacco budworm oviposition when sprayed onto TI 1112 plants (Jackson et al., 1984).

Other tobaccos, TI 1223 and TI 1341, with surface chemical compositions different from NC 2326, are also susceptible to tobacco budworm damage in the field (Johnson and Severson, 1984). TI 1223 produces the duvane diterpenes, α- and β-4,8,13-duvatrien-1-ols (α- and β-ols) and the labdane diterpene, *cis*-abienol. A mixture of duvanes, α- and β-ols and α- and β-diols, is present in the cuticular extractions of TI 1341 (Severson et al., 1984). In controlled cage studies, high levels of budworm ovipositional activity are observed on both TI 1223 and TI 1341 (unpublished data).

The research described herein was done to determine if duvane and labdane diterpenes stimulate tobacco budworm ovipositon when sprayed onto the budworm-resistant TI 1112. Docosanol and docosanyl myristate were also tested. Data from cage and field tests will be reported.

METHODS AND MATERIALS

The solvents used for plant extraction, component isolation, and spray application were Burdick and Jackson distilled-in-glass grade and were used as received. Docosanol [99+% by glass capillary gas chromatography (GC-2)] and myristic acid (99+ % by GC-2) were obtained from Sigma Chemical Company (St. Louis, Missouri).

Whole leaf cuticular components were extracted from tobacco grown under flue-cured conditions at Clemson University Pee Dee Research and Education Center, Florence, South Carolina; the Tobacco Research Station, Oxford, North Carolina; and the University of Georgia Coastal Plain Experiment Station, Tifton, Georgia, as described by Jackson et al. (1984). the NC 2326 WLW and MWS fractions were prepared from materials obtained in late May and early June at Tifton for that test year. The other diterpene components and mixtures were isolated from WLW extracts from various tobaccos obtained at all locations the previous crop year. All components and fractions were analyzed by GC-2 as described by Severson et al. (1984).

α- and β-Diol Mixture. NC 2326 MWS (2.5–3.0 g in $CHCl_3$) was placed into a 10-ml injection loop and was pumped onto a 2.5 × 58-cm liquid chromatography column (Laboratory Data Control) containing a 44-cm bed of Sephadex LH-20. Chloroform was pumped through the system at 2 ml/min, and 5-ml gel fractions (GF) were collected. After the collection of GF 45, the solvent was changed to 10% MeOH in $CHCl_3$ and pumped through the system for 3 hr. The column was reconditioned overnight using a 0.5 ml/min flow of $CHCl_3$. The fractions rich in diols (GF 35–42) were combined, and the solvent was removed from the sample on a rotary evaporator at 40°C under reduced pressure (100–150 mm Hg). The residue was placed in a desiccator under vacuum (~ 1 mm Hg) for 4 hr to yield 1.8–2.0 g of α- and β-diol mixture (71% α, 23% β by GC-2). The major impurities were oxidized diols (oxydihydroxy and trihydroxy duvanes).

α- and β-Ols. About 3 g of TI 1341 WLW in 10 ml of $CHCl_3$ was placed onto a Sephadex LH-20 column and eluted as above. Gel fractions rich in α- and β-ols (GF 25–32) were combined and the solvent removed. The residue was dissolved in hexane and placed on a 50-g basic alumina column (activity grade 1, slurry packed in hexane) and eluted with 0.5 liters of 1:3 CH_2Cl_2–hexane. The α- and β-ols were then eluted with 1.5 liters of 1:1 CH_2Cl_2–hexane. Solvent was removed as described above to yield about 650 mg of a colorless oil (98% by CG-2, α- to β-ol ratio 9:1).

α-Diol, β-Diol, cis-*Abienol, and Docosanyl Myristate.* α-Diol (mp 65–66°C, 99+ % by GC-2) (mp 65–66°C, Roberts and Rowland, 1962), β-diol (mp 122–123°C, 99+ % by GC-2) (mp 126°C, Roberts and Rowland, 1962), and *cis*-abienol, as a monohydrate (mp 63–65°C, with softening, 99+ % by GC-2) (mp 65%C, with prior softening, Gray and Mills, 1964) were isolated as described by Severson et al. (1982). Docosanyl myristate was prepared from docosanol and myristic acid (mp 55.5–56°C; *m/e* 536, C_{36} H_{52} O_2; 98+ % by GC-2).

Formulation of Spray Materials. A three-year average yield of about 55 mg of α- plus β-diols (α- to β-diol ratio approx 3:1) per plant was obtained from 6-week-old NC 2326 plants. Therefore, the materials for spray applications were formulated at the following one-plant equivalent levels: NC 2326 WLW, NC 2326 MWS, and α- plus β-diol mixture at 55 mg of α- plus β-diol (as determined by GC-2 analyses); α-diol, α- and β-ol mixture, and *cis*-abienol at 41.2 mg; and β-diol at 13.8 mg. Docosanol and docosanyl myristate were formulated at 2 mg/plant which was 10–20 times their average levels on NC 2326 in the field. The above were dissolved in hexane–CH_2Cl_2 (3:1) at a concentration of one plant equivalent per 0.5 ml. The solutions, including a solvent blank (SB) of hexane–CH_2Cl_2 (3:1), were divided into 2-ml lots into vials and stored at $-17.8°C$ in the dark until needed. The vials were brought to ambient temperature before spraying and quantitatively transferred with carrier solution (3:1 acetone–H_2O) to yield 40 ml of spray solution. One plant equivalent of material (10 ml of mixture) was sprayed onto each test plant.

Ovipositional Cage Bioassays. A screen cage bioassay for tobacco budworm oviposition on potted tobacco plants was used for tests during 1980–1983 at the Tobacco Research Station, Oxford, North Carolina. Ten 3-day-old female moths were released shortly before dark into each $2.4 \times 2.4 \times 2.0$-m screened cage located outside. Four test plants were placed in the corner opposite four control plants. The morning following each test all eggs were counted. Details of plant production, insect rearing, cage design, egg monitoring procedure, and spray application of cuticular isolates were described by Jackson et al. (1983, 1984). Spray treatments are given in Table 1.

Before individual analyses of variance for each experiment, data were transformed to a percentage of the total eggs counted per replication. Preference for egg laying on a particular treatment versus the control was evaluated by a paired *t* test.

To determine the efficiency of the spraying procedure, cuticular leaf chemical samples were taken from sprayed and unsprayed tobaccos. One 2-diam. leaf plug was taken from each plant as soon as they dried after spraying (day 0) and one and two days later. These leaf plugs were dipped eight times into 10 ml of CH_2Cl_2 in 20-ml scintillation vials. These samples were frozen immediately, shipped on Dry Ice to Athens, Georgia, and stored at $-17.8°C$. The cuticular chemical samples were analyzed by GC-2 as described by Severson et al. (1984).

Field Experiments. The activities of the cuticular diterpenes from tobacco on budworm oviposition were tested in the field at Oxford, North Carolina, and Florence, South Carolina, during 1983. Five replications of six treatments of 6-week-old tobacco plants were arranged in a cross pattern in fields isolated from other tobaccos. The six treatments were: TI 1112 with SB; TI 1112 with NC 2326 MWS; TI 1112 with α-diol; TI 1112 with β-diol; TI 1112 with α + β-ols; and NC 2326 with SB. The centers of adjacent replications were 10 m apart. Within a replication, the treatments were positioned at the points of an

TABLE 1. OVIPOSITION OF TOBACCO BUDWORM MOTHS IN SCREEN CAGES ONTO POTTED TOBACCO PLANTS SPRAYED WITH CUTICULAR COMPONENTS EXTRACTED FROM GREEN TOBACCO LEAVES, OXFORD, NORTH CAROLINA, 1980–1983

	Entry A			Entry B			
Experiment number[a]	Tobacco type	Spray treatment[b]	Amount applied (mg/plant)	Tobacco type	Spray treatment[b]	Amount applied (mg/plant)	Percent of eggs on entry A
1	NC 2326	None		TI 1112	None		75.2[d]
2	TI 1112	NC 2326 WLW	55.0[c]	TI 1112	SB		76.5[d]
3	TI 1112	NC 2326 MWS	55.0[c]	TI 1112	SB		74.8[d]
4	TI 1112	α-Diol	41.2	TI 1112	SB		64.2[d]
5	TI 1112	β-Diol	13.8	TI 1112	SB		63.3[d]
6	TI 1112	α + β-Diols	55.0	TI 1112	SB		72.2[e]
7	TI 1112	α + β-ols	41.2	TI 1112	SB		65.6[d]
8	TI 1112	α-Diol	41.2	TI 1112	NC 2326 WLW	55.0[c]	55.5
9	TI 1112	β-Diol	13.8	TI 1112	NC 2326 WLW	55.0[c]	59.8
10	TI 1112	α + β-Diols	55.0	TI 1112	NC 2326 WLW	55.0[c]	64.9[e]
11	TI 1112	α + β-ols	41.2	TI 1112	NC 2326 WLW	55.0[e]	50.7
12	TI 1112	α-Diol	41.2	TI 1112	NC 2326 MWS	55.0[e]	50.4
13	TI 1112	β-Diol	13.8	TI 1112	NC 2326 MWS	55.0[c]	46.0
14	TI 1112	α + β-Diols	55.0	TI 1112	NC 2326 MWS	55.0[c]	48.7
15	TI 1112	docosanol	2.0	TI 1112	SB		50.5
16	TI 1112	*cis*-abienol	41.2	TI 1112	SB		45.2
17	TI 1112	docosanyl myristate	2.0	TI 1112	SB		45.2

[a] Experiment 1 had 109 replications over a four-year period, experiments 2–17 had 8–27 replications, which averaged 20.5–55.1 eggs per plant.
[b] WLW = whole leaf wash; MWS = methanol–water-soluble fraction of WLW; α-diol = α-4,8,13-duvatriene-1,3-diol; β-diol = β-4,8,13-duvatriene-1,3-diol; α + β-ol = combinations of α- and β-4,8,13-duvatrien-1-ol; SB = solvent blank of 0.5 ml hexane–methylene chloride (3:1) in 9.5 ml acetone–water (3:1)
[c] Amount of α- + β-diol in the mixture.
[d] Significantly different, paired t test ($P < 0.01$).
[e] Significantly different, paired t test ($P < 0.05$).

equilateral hexagon with the nearest plants of different treatments being ca. 2.5 m apart. Four plants per treatment per replication were arranged in a square with 0.5 m between plants. The Oxford and Florence tests had similar designs, except that 6-week-old potted plants (two weeks in greenhouse and four weeks in shade outdoors) were used at Oxford and 6-week-old field-grown plants were used at Florence. One spraying per test was made by the same technique as described above for cage bioassays. The Oxford plants were sprayed starting at 1100 hr on August 23. After they dried, the plants were moved to a recently mowed stubble field of harvested wheat and arranged in the pattern described above. This field was over 500 m from the nearest tobacco field. Two hundred mated female budworm moths (40 per replication) were released per night for four successive nights. Eggs were counted and removed on four successive days.

The design at Florence was similar. Spraying of this test was begun at 1500 hr on July 7. However, after completion of these chemical applications, a large thunderstorm appeared. To prevent loss of this experiment, the plants were covered. A stake was driven into the ground in the center of each group of plants and they were covered with a tent of clear polyethylene. The rain prevented the release of moths that evening. The following morning the polyethylene was removed and a shade of a 1.2×1.2-m sheet of plywood was placed over each group of plants. These were removed just prior to moth release that evening. Therefore, day 0 for the Florence test was 24 hr after spray application. The field spray-back tests were sampled for chemical analyses as described above for cage tests.

Since the experimental designs were the same at the two locations, data from the Oxford and Florence tests were transformed to log $(x + 1.0)$ and combined prior to analysis of variance. Treatment means were separated by Duncan's new multiple-range test.

RESULTS

Ovipositional Cage Bioassays. As previously reported (Jackson et al., 1983, 1984), ca. 75% of the budworm eggs were deposited on unsprayed NC 2326 plants when moths were given a free choice between them and unsprayed TI 1112 plants (experiment 1, Table 1). Also, as previously shown (Jackson et al., 1984), both NC 2326 WLW and NC 2326 MWS were active in stimulating tobacco budworm oviposition onto TI 1112 plants (experiments 2 and 3, Table 1).

Tobacco budworm oviposition was significantly higher on TI 1112 plants sprayed with any of the individual duvanes or combinations of duvane isomers than it was on control TI 1112 plants sprayed only with the solvent blank (experiments 4–7, Table 1). This activity also persisted in experiments where duvane-treated TI 1112 were tested against control TI 1112 plants treated with NC 2326 WLW or NC 2326 MWS (experiments 8–14, Table 1). Neither docosanol, *cis*-abienol, nor docosanyl myristate stimulated budworm oviposition in the cage bioassays.

The levels of cuticular chemicals remaining on treated TI 1112 plants used in the cage bioassays declined over time after spraying. The α + β-4,8,13-duvatrien-1-ols and *cis*-abienol broke down most rapidly and significantly less (ca. 10%) of these compounds remained on the plants by two days after spraying (Table 2). Both the α- and β-4,8,13-duvatriene-1,3-diols applied individually or in NC 2326 MWS were more stable, with ca. 21–30% remaining after two days. Nearly 50% of the docosanol remained on the TI-1112 plants after two days (Table 2).

Field Experiments. All TI 1112 plants sprayed with duvane diterpenes or NC 2326 MWS had significantly ($P = 0.05$) more eggs deposited on them than TI 1112 plants sprayed only with solvent blank (Table 3). This trend continued for three days after spraying. Only the TI 1112 with NC 2326 MWS treatment had similar numbers of eggs as the NC 2326 control plants the first night after spraying. All of the treatments had significantly fewer eggs than NC 2326 by the second night after spraying.

However, as shown in Figure 1, all the diterpenes degraded rapidly in the field. This fast degradation explains the decrease in eggs found after the second night on these spray treatments relative to NC 2326. No changes were observed over time in the cuticular component profiles of NC 2326 and TI 1112 sprayed only with SB. The α- and β-ols disappeared at the fastest rate and were nearly absent two days after applications. The α- and β-diols were somewhat more stable, and they approached TI 1112 levels only after three days in the field.

DISCUSSION

Each of the duvanes tested in cage bioassays and field experiments stimulated tobacco budworm oviposition onto TI 1112 plants sprayed with these materials. None of the nonduvane cuticular components from tobacco increased egg laying. These data are evidence that the observed ovipositional nonpreference resistance of TI 1112 by *H. virescens* is due in part to the absence of duvane diterpenes which are major components of the leaf surface chemical profiles of commercial American tobaccos.

Our data indicate that β-diol may have a higher activity than the α isomer. The α:β ratio for NC 2326 MWS was ca. 3:1, and this ratio changed little over time (Table 2). Severson et al. (1984) reported an α:β ratio of 2.9:1.0 for NC 2326, and this ratio approximated 3:1 for 11 varieties of flue-cured, burley, Maryland, dark-fired, cigar wrapper, cigar binder, and Turkish tobaccos. Roberts and Rowland (1962) showed that α-diol is more heat and light labile than

TABLE 2. STABILITY OF CUTICULAR COMPONENTS SPRAYED ONTO TI 1112 TOBACCO PLANTS USED IN OVIPOSITION CAGE BIOASSAYS AT OXFORD, NORTH CAROLINA, 1983

Plant type	Spray treatment[a]	Components measured	Level ($\mu g/cm^2$) at days after application: 0	1	2
TI 1112	NC 2326 MWS	α + β-ols	0.3	0.1	Trace
		α-Diol	40.9	24.8	16.0
		β-Diol	15.3	9.4	6.1
TI 1112	α + β-ols	α-ol	60.7	25.7	7.5
		β-ol	6.6	2.9	0.9
TI 1112	α-Diol	α-Diol	50.5	29.4	17.9
		β-Diol	0.2	0.2	0.2
TI 1112	β-Diol	α-Diol	0.2	0.2	0.4
		β-Diol	15.0	7.9	3.2
TI 1112	*cis*-Abienol	*cis*-Abienol	66.4	11.3	6.1
TI 1112	Docosanol	1-Docosanol	2.7	1.9	1.2
NC 2326	None (check)	α-ol	0.1	0.1	0.1
		β-ol	0.1	0.1	0.1
		α-Diol	11.1	9.7	6.8
		β-Diol	4.5	3.8	3.1
		cis-Abienol	0.0	0.0	0.0
		1-Docosanol	0.2	0.2	0.1
TI 1112	None (check)	α-ol	0.0	0.0	0.0
		β-ol	0.0	0.0	0.0
		α-Diol	0.1	0.1	0.1
		β-Diol	0.1	0.1	0.1
		cis-Abienol	0.0	0.0	0.0
		1-Docosanol	0.0	0.0	0.0

[a] Materials applied in 0.5 ml of spray treatment per plant in 9.5 ml acetone–H_2O (3:1). MWS = methanol–water-soluble fraction of whole leaf wash; α + β-ols = α + β-4,8,13-duvatrien-1-ols; α-diol = α-4,8,13-duvatriene-1,3-diol; β-diol = β-4,8,13-duvatriene-1,3-diol.

TABLE 3. TOBACCO BUDWORM OVIPOSITION IN 1983 FIELD TESTS ON 6-WEEK-OLD TI 1112 OR NC 2326 PLANTS SPRAYED WITH DUVANE DITERPENES, METHANOL–WATER-SOLUBLE FRACTION OF NC 2326 WHOLE LEAF CUTICULAR WASH, OR SOLVENT BLANK

	Average number of eggs/plant on indicated day after spraying[b]		
Treatment[a]	1	2	3
TI 1112 with SB	4.9a	7.0a	7.7a
TI 1112 with β-Diol	7.7b	12.4b	11.8b
TI 1112 with α + β-ols	9.7bc	10.4b	19.4b
TI 1112 with NC 2326 MWS	15.9d	12.7b	12.3b
TI 1112 with α-Diol	12.4c	16.1b	21.3b
NC 2326 with SB	20.0d	25.4c	25.4c

[a] SB = solvent blank; α-diol = α-4,8,13-duvatriene-1,3-diol; β-diol = β-4,8,13-duvatriene-1,3,-diol; α + β-ols = mixture of α- and β-4,8,13-duvatrien-1-ols; NC 2326 MWS = methanol-water-soluble fraction from NC 2326 whole leaf wash.

[b] Data combined from Oxford, North Carolina and Florence, South Carolina; means in the same column followed by the same letter are not significantly different ($P = 0.05$); Duncan's multiple-range test.

β-diol. For our field tests we also saw a slightly more rapid decline of α-diol than β-diol when these materials were applied individually (Figure 1). When applied as part of the NC 2326 MWS these chemicals did not disappear as rapidly, but α-diol still declined more rapidly than β-diol. The α- and β-ols broke down very rapidly and were nearly gone by the second day after spraying (Figure 1).

The data indicate that the diterpenes are continuously synthesized and secreted onto the surface of the rapidly growing leaves, and then decompose (Table 2). They are believed to be converted to higher oxidation states by phytoxidation (Reid, 1975) which then further decompose to numerous volatile terpenes that may be important flavor and aroma components (Colledge et al. 1975; Enzell, 1977; Kawashima and Gamou, 1979; Wahlberg et al., 1977; Demole and Dietrich, 1977; Reid, 1979). Mass spectral analysis indicated that the oxidized duvanes are hydroxyepoxy, hydroxyoxy, and trihydroxy degradation products of the parent α- and β-ols and diols reported by Demole and Dietrich (1977), Enzell (1977), and Enzell and Walhberg (1980). A slight increase in the levels of the oxidized duvanes after three days in the field does not appear to account for all of the decreases in the parent duvanes. Thus, it would indicate the losses occur due to the formation of volatile components and/or degradation products that are not soluble in CH_2Cl_2 or are not GC volatile. The data presented here showed that the presence of duvane diterpenes is important in stimulating budworm oviposition. However, more studies are needed to determine

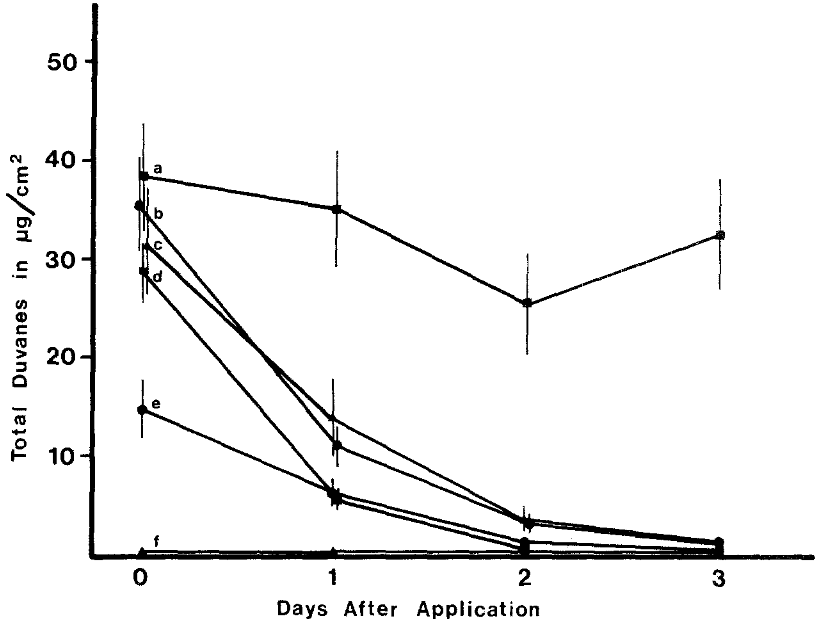

FIG. 1. Total duvane diterpenes on NC 2326 (a) and on TI 1112 plants sprayed with a solvent blank (f), the methanol–water-soluble fraction of NC 2326 whole leaf wash (c), α-4,8,13-duvatriene-1,3-diol (b), β-4,8,13-duvatriene-1,3-diol (e), or α- plus β-4,8,13-duvatrien-1-ols (d) in field tests with potted plants at Florence, South Carolina, and Oxford, North Carolina, 1983. Vertical lines indicate plus or minus standard error of the means; there were 10 samples per mean.

if oxidized duvanes or their volatile degradation products are also active in inducing budworm oviposition.

Acknowledgments—We thank J.S. Cheatham, J.M. Pitts, P.R. Granzow, and G. Carnell for assisting with the cage and field bioassays; K.L. McDuffie, L.B. Smith, and P.F. Mason for helping in extractions, purifications, and chemical analyses; and M.G. Stephenson for growing the tobacco at Tifton. Statistical assistance was provided by K.P. Burnham. This research was supported in part by a grant from the North Carolina Tobacco Foundation, Inc.

REFERENCES

CHAPLIN, J.F., BAUMHOVER, A.H., BURK, L.G., ELSEY, K.D., and MILES, J.D. 1976. Tobacco budworm resistance in *Nicotiana tabacum*. *Tob. Sci.* 20:156–157.

COLLEDGE, A., REID, W.W., and RUSSELL, R. 1975. The diterpenoids of *Nicotiana* species and their potential technological significance. *Chem. Ind.* 5(13):570–571.

DEMOLE, E., and DIETRICH, P. 1977. The chemistry of burley tobacco flavor (*Nicotiana tabacum* L.), pp. 1–36, *in* Recent Advances in the Chemical Composition of Tobacco and Tobacco Smoke. 173rd Am. Chem. Soc. Meet., Agric. Food Chem. Div., New Orleans, Louisiana. 592 pp.

ELSEY, K.D., and CHAPLIN, J.F. 1978. Resistance of tobacco introduction 1112 to the tobacco budworm and green peach aphid. *J. Econ. Entomol.* 71:723–725.

ENZELL, C.R. 1977. Recent progress in the chemistry of suncured tobaccos, pp. 37–77, *in* Recent Advances in the Chemical Composition of Tobacco and Tobacco Smoke. 173rd Am. Chem. Soc. Meet., Agric. Food Chem. Div., New Orleans, Louisiana. 592 pp.

ENZELL, C.R., and WAHLBERG, I. 1980. Leaf composition in relation to smoking quality and aroma. Program 34th Tobacco Chemists Research Conference, Oct. 27–29, 1980, Richmond, Virginia. Abstract No. 1, page 1.

ENZELL, C.R., WAHLBERG, I., and AASEN, A.J. 1977. Isoprenoids and alkaloids of tobacco. *Chem. Org. Naturst.* 34:1–79.

GRAY, P.S, and MILLS, J.S. 1964. The isolation of abienol from Canada Balsam, The oleoresin of *Abies balsamea* (L.) Mill. *J. Chem. Soc. Paper No. 1109, Suppl.* 1:5822–5825.

JACKSON, D.M, CHEATHAM, J.S., PITTS, J.M., and BAUMHOVER, A.H. 1983. Ovipositional response of tobacco budworm moths (Lepidoptera: Noctuidae) to tobacco introduction 1112 and NC 2326 in cage tests. *J. Econ. Entomol.* 76:1303–1308.

JACKSON, D.M., SEVERSON, R.F., JOHNSON, A.W., CHAPLIN, J.F., and STEPHENSON, M.G. 1984. Ovipositional response of tobacco budworm moths (Lepidoptera: Noctuidae) to cuticular chemical isolates from green tobacco leaves. *Environ. Entomol.* 13:1023–1030

JOHNSON, A.W. 1978. Evaluation of several *Nicotiana tabacum* entries for resistance to tobacco insect pests. *Tob. Sci.* 22:41–43.

JOHNSON, A.W., and SEVERSON R.F. 1982. Physical and chemical leaf surface characteristics of aphid-resistant and -susceptible tobacco. *Tob. Sci.* 26:98–102.

JOHNSON, A.W., and SEVERSON, A.W. 1984. Leaf surface chemistry of tobacco budworm-resistant tobaccos. *J. Agric. Entomol.* 1:23-32.

KAWASHIMA, N., and GAMOU, K. 1979. Studies on leaf surface lipid of tobacco II. Contribution of leaf surface lipid to aroma and taste of tobacco. *Utsunomiya Tab. Shikenjo Hokoku* 17:79–84.

MICHIE, M.J., and REID, W.W. 1968. Biosynthesis of complex terpenes in the leaf cuticle and trichomes of *Nicotiana tabacum*. *Nature* 218:578.

REID, W.W. 1975. Phytochemistry of the genus *Nicotiana*. Part IX. The diterpenes of *Nicotiana* as precursors of aroma constituent of commercial tobaccos. *Ann. Tabac., Sec. 2.* 12:33–38.

REID, W.W. 1979. The diterpenes of *Nicotiana* species and *N. tabacum* cultivars. *Linn. Soc. London. Symp. Ser.* 7:273–278.

REID, W.W. 1980. The diterpene chemistry of oriental tobaccos. Program of the 34th Tobacco Chemists Research Conference, October 27–29, 1980, Richmond, Virginia. Abstract No. 23., Page 12.

ROBERTS, D.L., and ROWLAND, R.L. 1962. Macrocyclic diterpenes. α- and β-4,8,13-duvatriene-1,3-diols from tobacco. *J. Org. Chem.* 27:3989–3995.

SATO, M., KOMARI, T., and ASAINE, N. 1982. Varietal differences in composition of leaf surface diterpenoids in tobacco. *Bull. Iwata Tob. Exp. Stn.* 14:59–70.

SEVERSON, R.F., MCDUFFIE, K.L., JACKSON, D.M., JOHNSON, A.W., STEPHENSON, M.G., and HERZOG, G.A. 1982. The fractionation of green tobacco leaf cuticular chemicals for insect bioassay studies. *Ga. J. Sci.* 40:15.

SEVERSON, R.F., GWYNN, G.R., CHAPLIN, J.F., and MILES, J.D. 1983. Leaf trichome exudate associated with insect resistance in *Nicotiana tabacum* L. *Tob. Sci.* 27:82–83.

SEVERSON, R.F., ARRENDALE, O.T., CHORTYK, O.T., JOHNSON, A.W., JACKSON, D.M., GWYNN, G.R., CHAPLIN, J.F., and STEPHENSON, M.G. 1984. Quantitation of the major cuticular components from green leaf of different tobacco types. *J. Agric. Food Chem.* 32:566–570.

WAHLBERG, I., KARLSSON, K., AUSTIN, D.J., JUNKER, N., ROERANDE, J., ENZELL, C.R., and JOHNSON, W.H. 1977. Effects of flue-curing and aging on the volatile, neutral and acidic constituents of Virginia tobacco. *Phytochemistry* 16:1217–1231.